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Sequence Analysis of HTLV-1 Provirus Associated With Adult T-Cell Leukemia/Lymphoma in Hong Kong

To the Editor: We have reported the first case of HTLV-1-associated adult T-cell leukemia/lymphoma (ATLL) in Hong Kong [1]. Now the sequence analysis of regions of *pX*, *pol*, and *env* (*gp21* and *gp46*) of the integrated provirus are discussed. The published sequences of these regions from Japanese prototype (ATK) and Papua New Guinea isolate (PNG-1) are also included for comparison (Fig. 1). The prototype ATK, an integrated provirus from a Japanese patient's leukemic cell, is the first to be completely sequenced [2]. PNG-1 is a newly characterized proviral clone from Papua New Guinea [3] and is thought to be evolutionally distinct from the prototype ATK.

High-molecular-weight DNA was extracted from the whole blood of the 42-year-old patient diagnosed with ATLL. The regions of interest were amplified by polymerase chain reaction (PCR). The PCR products were purified and sequenced directly with appropriate primers. The sequence of *pX* region amplified from our Hong Kong patient was virtually identical to published sequences of ATK and PNG-1. Since the open reading frame *pX* DNA encodes two important functional proteins, Tax and Rex, for viral expression and replication, the minimal sequence variation in this region among the HTLV-1 isolates is rather expected.

It is known that some regions within the *pol* gene are strongly conserved among different HTLV-1 clones because the protein it encodes is important in the retroviral life cycle. The sequence of the *pol* gene we amplified was identical to that of the published Japanese ATK but differed from the Papua New Guinea PNG-1 by 11 base-pairs. On the other hand, the sequence of the same region from PNG-1 varied by 9.3% from that of the prototype ATK [3].

The sequence similarity between the Japanese prototype ATK and the Hong Kong counterpart was also found in the *env* gene. The regions of the *env* gene we amplified included parts of *gp21* and *gp46* genes, which encode the transmembrane protein and the extracellular glycoprotein, respectively. The sequencing result of the amplified regions of the *gp21* and *gp46* gene showed a difference of only 5 and 3 base-pairs from those of the Japanese prototype ATK, whereas it showed a difference of 14 and 22 base-pairs from Papua New Guinea PNG-1.

The nucleotide sequence from our ATLL patient seemed more closely related to that of the Japanese. Furthermore, due to the geographic proximity and the fact that Hong Kong is not endemic of HTLV-1 infection, the integrated provirus from our Hong Kong patient might have originated from Japan.

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POL
CCTCCCTTGCTATTCGCCATAC*****************  HK
                                          ATK
                                          PNG-1

***********AGGACTTGTAGAACGCTCTAATGGCATT  HK
           --------------------------     ATK
           ---------G------------------   PNG-1

CTTAAAACCCTATTATATAAGTACTTTACTGACAAACCCG  HK
---------------------------------------  ATK
--------TT-----------------C----G----A   PNG-1

ACCTACCCATGGATAATGCTCTATCCATAGCCCTATGGAC  HK
---------------------------------------  ATK
-------T-----G-----------------T-------  PNG-1

AATCAACCAC**********************CAAAACC  HK
----------                                ATK
---T------                                PNG-1

CGATGCGAGCTTCACCAC                        HK

GP21
CAGTATGCTGCCCAGAACAGACG***********CCTGT  HK
                                    -----  ATK
                                    -----  PNG-1

TCTGGGAGCAAGGAGGATTATGCAAAGCATTACAAGAACA  HK
---------------------------------------  ATK
-------A-----------------G--GC---------G  PNG-1

GTGCTGTTTTCCGAATATTACTAATTCCCATGTCTCAATA  HK
----C----------------C-----------C-----  ATK
----T------TA--C--C--T--------CC--TT-----  PNG-1

TTACAAGAACGACCCCCCCTTGAGAATCGAGTCCTGACTG  HK
C--------A-------------------------------  ATK
-------------A---------------------------  PNG-1

GCTGGGGCCT**************CCTCTCACAGTGGGC  HK
----------                                ATK
--------T-                                PNG-1

TCGAG                                     HK

GP46
GGGTAAGTTTCTCGCCACTTTG*****************  HK
                                          ATK
                                          PNG-1

***CCCCTCATCTTCGGTGATTACAGCCCCAGCTGCTGTA  HK
   -----------------------------------   ATK
   -----AT---CA-T-C#------T-------------  PNG-1

CTCTCACAATTGGAGTCTCCTCATACCACTCTAAACCCTG  HK
---------------------------------------  ATK
-------T-----G---------------------------  PNG-1

CAATCCTGCCCAGCCAGTTTGTTCGTGGACCCTCGACCTG  HK
---------------------------------------  ATK
--------------------C--A--------T-----T  PNG-1

CTGGCCCTTTCAGCAGATCAGGCCCTACAGCCCCCCTGCC  HK
---------------------------------------  ATK
---------------------------------------  PNG-1

CTAACCTAGTAAGTTACTCCAGCTACCATGCCACCTATTC  HK
---------------------------------------  ATK
---GT-----G--C--------A------------------  PNG-1

CCTATATCTATTCCCTCATTGGATTAAAAAGCCAAACCGA  HK
----------------------C---G------------  ATK
------------------------T---A------------  PNG-1

AATGGCGGAGGCTATTATTCAGCCTCTTATTCAGACCCTT  HK
---------------------------------------  ATK
--------------C-----G--------------------  PNG-1

GTTCCTTAAAGAGCCCATACCTGGGGTGCCAATCATGGAC  HK
----------T-----------------------------  ATK
--------------------C---------------------  PNG-1

CTGCCCCTATACAGGAGCCGTCTCCAGCCCCTA         HK
--------                                  ATK
--------                                  PNG-1
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Fig. 1. Partial sequences of the amplified *pol*, *gp21*, and *gp46* regions. The published sequences of ATK and PNG-1 are aligned for comparison. The primer sequences for PCR and sequencing reaction are underlined. The region where the nucleotide sequence was not determined due to the sequencing limitation is indicated by an asterisk (*). A base deletion for the *gp46* of PNG-1 is represented by a number sign (#).

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Management of Iron Overload With a Disposable Multi-Day Delivery System

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To the Editor: Iron overload remains the most serious complication of long-term blood transfusions in patients with thalassaemia major and other transfusion dependent anemias. Regular desferrioxamine administration has been shown to prevent iron-related cardiac disease [1] and to extend survival in transfused patients [2]. In many patients, compliance to subcutaneous desferrioxamine therapy is poor because preparation of the solution is demanding and the nightly administration by syringe pump is irritating. Therefore, the prevention of iron-related morbidity remains a serious problem for many patients. Some centres have reported success with intensive intravenous iron chelation for patients with severe iron overload with, or at risk of, cardiac disease [3,4]. Because the ambulatory long-term intravenous therapy can provoke quality of life modifications for discontinuous scholastic and/or working activity, we provided domiciliary nursing assistance for the patients involved in our study.

Five thalassaemic patients, aged 12–15, were treated between June and December 1994 with a desferrioxamine dose of 40–60 mg/Kg/24 hr for 5 consecutive days, weekly for 26 consecutive weeks. The drug solution was prepared in hospital in a multi-day elastomeric disposable pump, the LV2 Infusor (Baxter, Deerfield, IL), with a reservoir volume of 250 ml. The Infusor was connected to a Port-a-Cath (SIMS-Deltec, Minneapolis, MN) placed on the anterior chest wall. The compliance with this protocol was considerably better than with the conventional treatment protocol. Patients commented favourably on the lightness and size of the device as well as its silence and simple operation. In addition, by eliminating the patient's responsibility for preparing their desferrioxamine solution, and by providing a domiciliary nursing service to assist with the administration, compliance was significantly improved with a corresponding reduction in ferritin levels. Before treatment, serum ferritin ranged from 3,000 to 5,100 mcg/l (mean 3840 ± 850 SD). Following 26 weeks of therapy, values ranged from 450 to 1,000 mcg/l (mean 668 ± 228 SD), a mean decrease of 82.8% (range 80–85%).

Drs. Fielding and Wonke [5] have used a disposable single-day elastomeric pump for intravenous desferrioxamine infusion. In contrast, we have introduced a multi-day elastomeric pump with the addition of domiciliary nursing assistance to reduce the risk of infection from faulty aseptic techniques in young children. Using the domiciliary support, no infectious complications were reported. All patients have expressed satisfaction with the domiciliary protocol because their scholastic activities were not affected and their quality of life was improved. The economics of this method of treatment were compared to the conventional pump, with net savings observed in favour of the elastomeric pump.

In summary, we conclude that the disposable multi-day pump is eminently suitable for long-term intravenous chelation therapy in young, iron-overloaded thalassaemic patients.

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Concurrent Epiphyseal Fracture and Leukemia in a Patient Treated With Growth Hormone

To the Editor: Pituitary growth hormone (GH) has been used as a therapeutic agent for the GH-deficient child for more than 30 years. The recent availability of biosynthetic GH has favored its use in a number of so-called "nonconventional" indications, with the expectation that all cases will be treated effectively and safely.

However, the literature raises growing concern about untoward adverse effects and oncogenic potential of GH. Until now, leukemia has been observed in at least 33 patients who had received both pituitary and recombinant GH in moderate doses [1,2]. There was a mean time of 5 years between start of GH therapy and onset of leukemia. Despite the number of case-reports, definite epidemiological evidence of a relationship between GH treatment in pediatric patients and leukemia or other malignancies is still lacking.

Nevertheless, there is some in vitro evidence in both animal models and humans suggesting a role of GH in hemopoietic disorders. GH enhances the proliferation of human leukemic blasts freshly obtained from acute leukemia (both lymphoid and myeloid types) [3]. GH also enhances granulocytic colony growth through a pathway involving insulin-like growth factor/somatomedin C [4].

We recently observed acute lymphoblastic leukemia in an 18-year-old white male, treated with recombinant GH for pituitary dwarfism. GH therapy was started at age 14 years and was continued for 34 months, resulting in growth acceleration. Fourteen months after GH discontinuation, the patient suffered from an epiphyseal fracture of the right tibia. The fracture was apparently related to a modest trauma. Six months later he developed a recurrent epiphyseal fracture of the right tibia, and he was admitted to hospital for orthopedic surgery. Hemogram at admission showed marked leukocytosis and presence of blast cells. Diagnosis of acute lymphoblastic leukemia pre-B phenotype FAB L2 was made, and the patient underwent induction chemotherapy.

Our case increases the number of reported malignancies following GH use. No additional leukemia risk factor could be assessed. The latency period between GH treatment and leukemia onset falls within limits observed in the literature. Clinical features at onset of leukemia in this case are impressive; in fact, pathological fractures are also extremely rare in pediatric leukemia patients (7% or less) and generally involve the spine [5]. It is interesting to note that osteoclasts may derive from pluripotent hemopoietic